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Antimicrobial Activity of Alcoholic German Chamomile Extract Against Oral Pathogens Including *Streptococcus mutans*, *Lactobacillus acidophilus*, and *Candida albicans*

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ABSTRACT

Resistance to common antiseptics has driven interest in plant-based alternatives to address recurring oral infections caused by bacterial and fungal pathogens. The antimicrobial activity of alcoholic German Chamomile extract was evaluated using agar well diffusion and minimum inhibitory concentration, minimum bactericidal concentration, and minimum fungicidal concentration (MIC/MBC/MFC) assays against ten isolates each of *Streptococcus mutans*, *Lactobacillus acidophilus*, and *Candida albicans*. GC–MS analysis identified key phytochemicals such as α-bisabolol, apigenin, and chamazulene in the ethanolic extract. The antimicrobial potential was tested by the agar well diffusion method using 10, 20, 30, and 40% concentrations with 0.2% chlorhexidine as a positive control. The extract inhibited all of the tested microbes in a concentration-dependent manner and was most effective against *C. albicans*, followed by *S. mutans* and *L. acidophilus*. One-way analysis of variance (ANOVA) confirmed significant differences ($p < 0.05$) at extract concentrations of 20–40% among microbial responses. Inhibition zones were comparable to those of chlorhexidine, confirming the extract's potency. MIC and MFC values for *C. albicans* were as low as 2.5% and 1.25%, respectively. In contrast, *S. mutans* showed moderate sensitivity to the extract, with MIC and MFC values of 5.0% and 2.5%, respectively. *L. acidophilus*, however, required higher concentrations to achieve full inhibitory and fungal effects, with MIC and MFC values of 10.0% and 5%, respectively, suggesting a higher relative resistance of this bacterial species to the extract. The extract showed pronounced antifungal and antibacterial effects at specific concentrations, with efficacy comparable to chlorhexidine. These effects are attributed to the presence of multiple bioactive compounds. Accordingly, Chamomile extract represents a promising natural candidate for the development of safe and effective alternatives to conventional chemical antiseptics in oral care, although further clinical and pharmaceutical investigations are required to validate its safety and therapeutic potential.

1. INTRODUCTION

Microorganisms such as bacteria and fungi, among others, are all causative agents of diseases/conditions found within or affecting the mouth and oral cavity. When the balance of the oral microbiome is disrupted, pathogenic microorganisms proliferate at the expense of symbiotic and beneficial microorganisms, leading to an imbalance in the natural microbial balance in the mouth and contributing to the development of various oral diseases [1, 2]. Because of their prevalence and chronic nature, oral diseases like Dental Carries and Oral Candidiasis represent significant public health concerns globally. There is an abundance of research that indicates that the makeup of your Oral Microbiome is critical to oral health and general health, as many microorganisms from the mouth can influence the inflammatory environment of the Host [3]. Oral infections may be linked to the following diseases/disorders: Rheumatoid Arthritis, Nephritis, Endocarditis, and others, which are primarily inflammatory in nature [4]. Dental Caries have long been attributed to *S. mutans,* and research strongly supports this assertion. The primary cariogenic potential of *S. mutans* stems from its ability to ferment carbohydrates (dietary sugars) through the production of organic acids, which creates enamel demineralization and subsequently the development of caries. In addition to the aforementioned, *S. Mutans* also possesses other virulence factors such as Polysaccharides and Glucosyltransferase enzymes that allow for adhesion to tooth surfaces, as well as supporting the overall structure of Dental Biofilms. Establishing a healthy microbiome is essential to

preventing the establishment of disease and the presence of pathogenic species. The formation of microbial biofilms increases the resistance of bacteria to conventional antimicrobial agents and chemical plaque control. The ability of *S. mutans* to grow at a low pH (its aciduric capacity) contributes to its dominance in cariogenic environments. As a result, much research on *S. mutans* has concentrated on it as a principal target for antimicrobial agents, vaccines, and probiotic therapies [5]. Traditionally, the identification of *S. mutans* has relied upon phenotypic characteristics such as colony morphology on selective media, for example, mitis salivarius agar, and biochemical assays, including the carbohydrate fermentation test. However, molecular diagnostic approaches to identification are increasingly being used to enhance the specificity and accuracy of identification [6]. *Lactobacillus acidophilus*, a member of the lactic acid bacteria group, has gained increased interest as a potential oral probiotic and as a modulator of the oral microbiome. It has been demonstrated that certain strains of *L. acidophilus* can inhibit the growth of major oral pathogens (e.g., *S. mutans* and periodontal bacteria) and prevent or disrupt the adhesion of pathogens and biofilm formation, as well as reduce levels of inflammatory markers when administered with standard therapies [7, 8]. These benefits have largely been attributed to the production of bacteriocins, hydrogen peroxide, and organic acids. According to research, the antimicrobial effects of *L. acidophilus* are likely due to its production of bacteriocins, hydrogen peroxide, organic acids, and its competition with other microbes for binding sites on mucosal surfaces [9]. It has also been suggested that *L. acidophilus* may enhance innate immune responses by increasing IgA production in saliva and altering cytokine production [10]. However, although this is very promising research, there is still some disagreement on whether *L. acidophilus* should be used as an oral probiotic. Some strains of *L. acidophilus* produce large amounts of acid that could potentially reduce the pH of the mouth and result in demineralization of tooth enamel or cavities if not formulated carefully [11]. Therefore, when considering the application of *Lactobacillus acidophilus* as an oral probiotic, special attention should be given to strain specificity, safety assessment, and rigorous clinical evaluation. It will also be necessary to perform more randomized controlled trials to determine which strains are the most effective, the best delivery methods, and the appropriate dosages needed to maximize beneficial effects and minimize risks to dental health [8, 9]. *Candida albicans* is another opportunistic fungal microorganism that can be found in the mouth among other microorganisms. In a healthy individual, Candida is normally found in small numbers, but many factors can disrupt the balance of microorganisms in the mouth, resulting in an increased amount of Candida in the oral cavity. These factors include compromised immune function (e.g., HIV), dry mouth (xerostomia), uncontrolled diabetes, prolonged denture use, the use of broad-spectrum antibiotics, and the use of corticosteroids. The emergence of Oral Candidiasis (also known as Thrush) is a condition that can result from the overgrowth of oral cancer, and there are many presentations of clinical Candidiasis, including pseudomembranous and hyperplastic. The identification of *C. albicans* can be performed by direct microscopy as well as by culture on nutrient media (e.g., Sabouraud Dextrose Agar (SDA)) and by biochemical identification and molecular methods [12]. The modern-day trend of using medicinal plants for research purposes represents a chance to discover natural and safe alternatives to traditional chemical agents. German Chamomile (*Matricaria chamomilla*), whose potential benefits come from its wealth of bioactive materials as well as its ability to inhibit bacterial growth, is a significant focus of this study because of this potential. German Chamomile is composed of many flowers that have an appearance very similar to Daisies. It is used as a popular herbal medicine due to its potential for having various types of chemical properties, including anti-inflammatory, antioxidant, and antimicrobial properties and is therefore very popular in Herbal Medicine and Aromatherapy [13]. Studies [13, 14] conducted over the past few years have also investigated the efficacy of German Chamomile in the control of many microorganisms, specifically bacteria responsible for causing oral infections (e.g., *S. mutans*, *P. gingivalis*, and *C. albicans*). Some medicinal properties of Chamomile are due to its active compounds: flavonoids and terpenoids, with Chamazulene as the primary active compound found in Chamomile essential oil. Chamomile has been used for centuries as an herbal remedy for multiple ailments, including digestive disorders, anxiety, insomnia, and skin conditions. Many people use the soothing properties of Chamomile in tea, tinctures (herbal preparations), and topical creams. Some scientific studies have shown the ability of Chamomile to prevent the formation of oral bacterial biofilms and to inhibit the growth of some oral bacteria. Several laboratory experiments using techniques such as Disk diffusion assays, minimum bactericidal concentration (MBC) tests, and minimum fungicidal concentration (MFC) tests have shown that Chamomile extracts are effective against several types of oral microorganisms compared to the standard bacteriostatic agent, chlorhexidine. Due to the biocompatibility and natural source of Chamomile extracts, it is a good candidate for use in products such as mouthwashes, toothpaste, and other products used for oral health [15-17]. While there have been some studies on the antimicrobial properties of Chamomile, they have mainly focused on studying the effects of different dosages and comparing their effects to standardised treatments for preventing infection with particular bacteria. There have not yet been any studies that have looked at how effective different concentrations of Chamomile's alcoholic extracts are against a particular pathogen when compared directly to other forms of treatment. Limited data remain to determine the effectiveness of these extracts against pathogenic strains associated with oral infections, including some beneficial species such as *Lactobacillus acidophilus* or other probiotic bacteria. This research project aims at identifying chemicals present in the extract and analyzing the active substances that contribute to these extracts' antimicrobial activities, using gas chromatography-mass spectrometry (GC-MS). Additionally, the objective of this study is also to determine the MIC/MBC/MFC of alcoholic extract of Chamomile in comparison to 0.2% concentration of chlorhexidine as common antimicrobial treatment effects, bactericidal and fungicidal ethanolic extract concentrations, as well as direct comparisons with clinical gold standards, are still insufficient, particularly with regard to specific groups of oral pathogens, including the probiotic *L. acidophilus*. The study also aimed to analyze the phytochemical composition of the Chamomile extract using GC-MS to identify the active compounds responsible for its antimicrobial properties.

2. MATERIAL AND METHODS

2.1 Microbial Isolates

The ten known isolates of *S. mutans*, *L. acidophilus*, and *C. albicans* were sourced from the microbiology lab of the College of Dentistry, University of Baghdad; all were obtained from the mouths of patients who had been attending the hospital. Identification was done using morphological characteristics on specific media, Gram's stain [18], and Vitek 2 tests (bioMérieux, France). The *S. mutans* isolates were cultured on Mitis Salivarius Bacitracin agar (MSBA) and incubated at 37℃ for 48 h in 5% CO_2. *Lactobacillus acidophilus* isolates were grown on Rogosa agar under anaerobic conditions at 37℃ for 48 h, while *Candida albicans* isolates were cultivated on SDA and incubated at 37℃ for 24–48 h.

2.2 Activation of isolates

Pure colonies of each organism were sub-cultured on Brain Heart Infusion Agar (BHIA; HiMedia, India) and incubated at 37℃ for 24 h. A single colony from each culture was inoculated into 10 mL of sterile Brain Heart Infusion Broth (BHIB; HiMedia, India) and incubated aerobically at 37℃ for 24 h to achieve a turbidity equivalent to 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL) [19].

2.3 Preparation of alcoholic Chamomile extract

Dried flowers of German Chamomile were subjected to cold maceration [20]. A total of 500 g of powdered material was soaked in 2 L of 70% ethanol (Chem-Lab, Belgium) for three days at room temperature, 25℃, with continuous magnetic stirring. The mixture was filtered successively through sterile gauze and Whatman No. 1 filter paper (UK). The filtrate was concentrated under reduced pressure using a rotary evaporator at 45℃, yielding a concentrated extract that was stored in a dark container at 4℃ until use. The extraction yield was calculated:

$$\text{Yield (\%)} = [\text{Weight of dried extract (g)} / \text{Weight of plant sample (g)}] \times 100$$

2.4 Gas Chromatography–Mass Spectrometry (GC–MS) analysis

In cooperation with the Environmental Research Center, Department of Water and Environment, Ministry of Science and Technology, a Shimadzu GC-MS system (model QP2010 Plus) was used to analyze the phytochemical makeup of German Chamomile extract. The extract was filtered and concentrated before being put into the GC-MS system.

The alcoholic extract of Chamomile was analyzed using GC-MS to identify the active chemical compounds. An HP-5MS column (30 m long × 0.25 mm inner diameter × 0.25 µm coating thickness) was used.

The oven temperature program started at 60℃ and held for 2 minutes. The temperature was then increased at a rate of 5℃/min to 250℃ and held for 10 minutes. The injection port temperature was set at 250°C and the detector temperature at 280℃, with a helium carrier gas flow rate of 1.0 mL/min. A 1:20 split injection pattern and a 1 µL injection volume were used.

The compounds were identified by matching the mass spectra with the TIC Mass Spectral Library database to ensure accurate component identification [21].

2.5 Antimicrobial susceptibility testing

The antimicrobial activity of the alcoholic Chamomile extract was assessed using the agar-well diffusion technique against *S. mutans*, *L. acidophilus*, and *C. albicans*. Extract solutions were formulated at final concentrations of 10%, 20%, 30%, and 40%. Chlorhexidine (CHX) at 0.2% served as the positive control.

Mueller–Hinton agar plates were inoculated with 100 µL of standardized microbial suspension (≈ 1.5×10^8 CFU/mL). Wells (6 mm diameter) were made aseptically using a cork borer, and each was filled with 100 µL of extract at the designated concentration. Inhibition zones were measured after incubation at 37℃ for 24 h using a ruler. Absence of an inhibition zone indicated microbial resistance to the tested concentration [22].

2.6 Determination of MIC, MBC, and MFC

Following sterilization, the culture medium was allowed to cool to a molten state. Before solidification, the molten medium was distributed into sterile Petri dishes, each containing a predetermined concentration of the alcoholic extract (10, 5, 2.5, 1.25, 0.625, and 0.312%) with the final volume adjusted to 10 mL per plate (Table 1). A negative control plate containing only BHIA and microbial suspension was included, while experimental plates contained BHIA supplemented with varying extract concentrations. The plates were gently swirled in a circular motion to ensure uniform distribution of the extract within the medium and then allowed to solidify at room temperature. Each solidified plate was surface-inoculated with 100 µL of the microbial suspension and evenly spread using a sterile spreader. The inoculated plates were incubated at 37℃ for 24 hours. Microbial growth was evaluated by visual observation of colony formation on the agar surface. The MIC was defined as the lowest concentration that showed few colony growth, while the MBC and MFC were defined as the lowest concentration of the extract that resulted in complete inhibition of visible growth, indicating total microbial lethality [23].

Table 1. The final extract concentrations with BHIA

Volume of BHI.A Medium (mL)	Volume of Extract (mL)	Desired Concentration (%)
9	1	10
9.5	0.5	5
9.75	0.25	2.5
9.844	0.156	1.56
9.875	0.125	1.25
9.9375	0.0625	0.625
9.968	0.0312	0.312

2.7 Statistical analysis

All statistical analyses were conducted using SPSS software, version 24.0 (IBM Corp., Armonk, NY, USA), while graphical illustrations and figures were prepared using Microsoft Excel, version 10.0. Descriptive analyses included the determination of mean and standard deviation (SD) to summarize and

characterize the dataset. Comparative statistical tests were applied to evaluate differences among groups. A Student's t-test was employed to compare means between two groups, while an F-test using one-way analysis of variance (ANOVA) was performed to assess variations among multiple groups [24].

3. RESULTS AND DISCUSSION

The recovered isolates of *S. mutans, L. acidophilus*, and *C. albicans* were confirmed by their characteristic growth and microscopic features. *S. mutans* grown on MSBA formed light blue, rough, raised colonies measuring 1–2 mm in diameter. Gram staining revealed Gram-positive cocci arranged in short or long chains (VITEK 2 identification, BioNumber: 100011564753531). *Lactobacillus acidophilus* on Rogosa agar produced grayish-white colonies, 0.5–2.5 mm in diameter, and Gram-positive rod-shaped bacteria, sometimes arranged in chains. (VITEK 2 identification, BioNumber: 3377700410001). *Candida albicans* grew on SDA as smooth, creamy, pasty colonies with oval budding cells under the microscope (VITEK 2 identification, BioNumber: 4112566065327771) [25, 26].

3.1 Extraction yield

The yield of the alcoholic extract from 500 g of dried Chamomile flowers was 76.56 g, representing 25.52% (w/w). The extract appeared as a dark yellowish-brown residue with aromatic odor, stored at 4°C for the subsequent testing.

3.2 GC–MS phytochemical profile

Gas Chromatography–Mass Spectrometry analysis (GC-MS) of Chamomile extract revealed a broad spectrum of biologically active compounds belonging to several chemical classes, including lactones, fatty acids, unsaturated alcohols, phenolic compounds, and spiroethers. High intensity peaks were recorded, reflecting the relative abundance of certain compounds known for their antimicrobial and antifungal (Table 2, Figures 1 and 2).

Table 2. Major compounds identified by GC–MS

Peak No.	Identified / Reassigned Compound (Literature-based)	Retention Time (min)	Area (%)	Chemical Class
34	α-Bisabolol–related sesquiterpene derivative	31.99	23.35	Terpenoid alcohol
52	Long-chain unsaturated fatty alcohol	41.15	17.41	Unsaturated fatty alcohol
31	Coumarin derivative (7-methoxy coumarin-like)	31.32	7.56	Lactone / Coumarin
45	Ethyl hexadecanoate	37.71	7.14	Fatty acid ester
42	Palmitic acid (n-hexadecanoic acid)	36.91	6.68	Saturated fatty acid
53	Linoleyl alcohol derivative	41.76	6.57	Fatty alcohol
40	Chamomile-type spiroether derivative	35.34	6.16	Spiroether
54	Long-chain acetylenic alcohol	41.90	4.90	Fatty alcohol
41	Minor spiroether derivative	35.55	2.23	Spiroether
51	Anhydrosugar derivative	23.75	2.05	Carbohydrate derivative

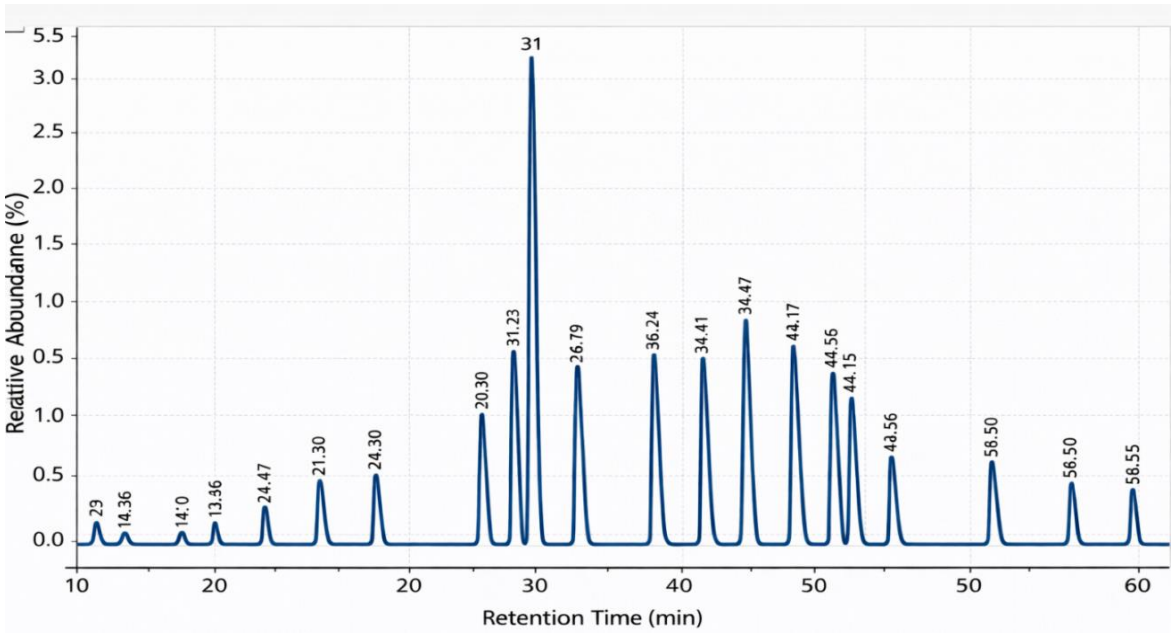

Figure 1. Chromatogram Matricaria extract

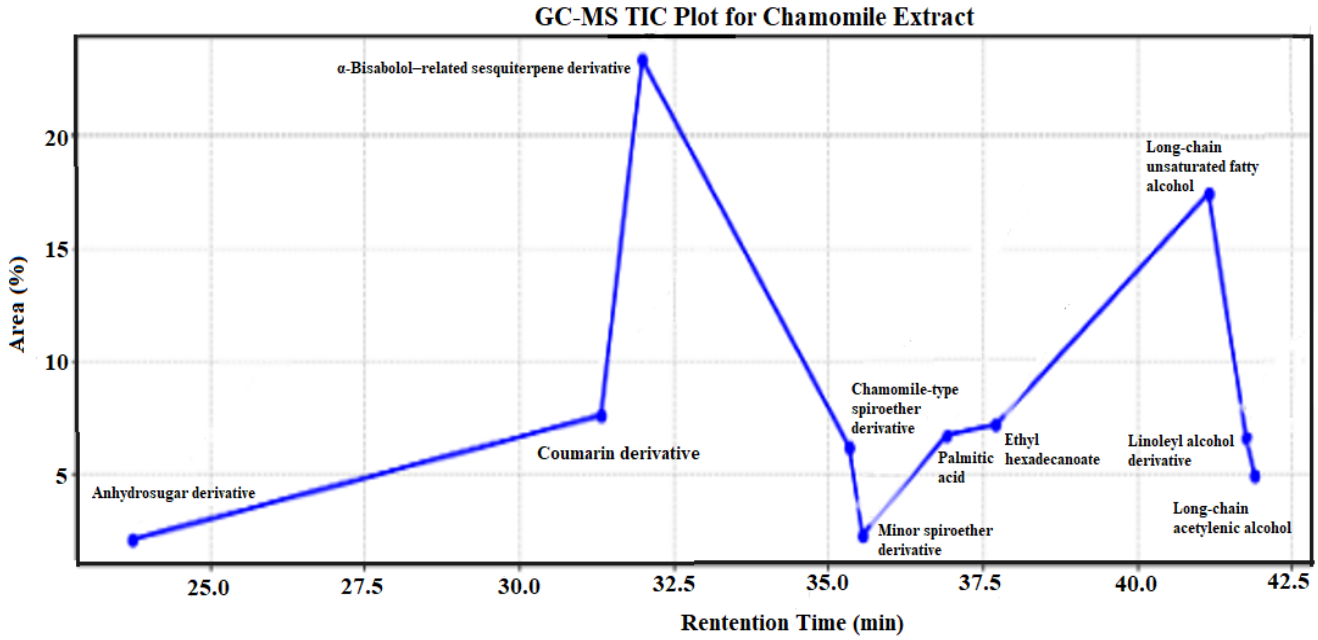

Figure 2. GC-MS plot for Chamomile extract

The most notable findings included the predominance of Spiroether derivatives (1,6-Dioxaspiro[4,4]non-3-ene derivatives), which are characteristic compounds of Chamomile and strongly associated with its antifungal activity, particularly against *Candida albicans*. Coumarin derivatives, such as (2H-1-Benzopyran-2-one,7-methoxy-), are known for their antimicrobial and antioxidant properties. Long-chain alcohols and fatty acids (such as hexadecanoic acid and its derivatives), which contribute to the inhibitory effect on bacteria by affecting cell membrane permeability. This chemical diversity and relatively high abundance of active compounds support the biological findings, which demonstrated clear efficacy of the extract against *C. albicans*, moderate susceptibility to *S. mutans*, and relatively higher resistance to *L. acidophilus*.

3.3 Antimicrobial activity of the alcoholic Chamomile extract

The antimicrobial potential of the alcoholic extract of Chamomile was assessed against *S. mutans, L. acidophilus*, and *C. albicans* using the agar well diffusion method at different concentrations (10%, 20%, 30%, and 40%), with 0.2% chlorhexidine (CHX) as the positive control. The mean values and standard deviation (SD) values of the inhibition zones in millimeters (mm) of the German Chamomile against the bacteria are presented in Tables 1-3. The alcoholic extract of German Chamomile demonstrated a clear concentration-dependent antimicrobial activity against all tested microorganisms.

Streptococcus mutans

As presented in Table 3, the inhibition zone increased significantly, for *S. mutans* isolates, from 12.30 ± 0.45 mm at 10% extract to 24.40 ± 0.32 mm at 40%, showing nearly equal efficacy to 0.2% chlorhexidine (24.60 ± 0.30 mm). This strong antibacterial effect can be attributed to Chamomile's flavonoids (apigenin, luteolin) and terpenoids (α-bisabolol, chamazulene), which disrupt bacterial membranes and inhibit glucosyltransferase enzymes responsible for biofilm formation.

Table 3. Inhibition zones (Mean ± SD) for *Streptococcus mutans* at different Chamomile extract concentrations

Concentration (%)	No. of Isolates	Mean ± SD (mm)	Significance vs CHX
10%	10	12.30 ± 0.45	—
20%	10	16.80 ± 0.40	$p < 0.001$
30%	10	20.50 ± 0.38	$p < 0.001$
40%	10	24.40 ± 0.32	NS ($p > 0.05$)
CHX 0.2%	10	24.60 ± 0.30	Reference

CHX = chlorhexidine; SD = standard deviation. Statistical significance: $p < 0.05$ = significant (S); $p < 0.001$ = highly significant (HS); NS = not significant.

Lactobacillus acidophilus

The results for *Lactobacillus acidophilus* isolates, shown in Table 4, exhibited a similar inhibition pattern, demonstrating moderate sensitivity that gradually increased with rising extract concentration. The average inhibition zone expanded from 10.20 ± 0.45 mm at a 10% concentration to 22.10 ± 0.35 mm at a 40% concentration. The relatively lower sensitivity compared to *S. mutans* may indicate a higher resistance of lactobacilli to plant phenolic compounds. Nevertheless, the antimicrobial activity was close to that of chlorhexidine, supporting the potential use of Chamomile extract as an effective natural ingredient in oral care products such as mouthwash.

Table 4. Inhibition zones (Mean ± SD) for *Lactobacillus acidophilus* at different Chamomile extract concentrations

Concentration (%)	No. of Isolates	Mean ± SD (mm)	Significance vs CHX
10%	10	10.20 ± 0.45	—
20%	10	14.30 ± 0.60	$p < 0.001$
30%	10	18.40 ± 0.52	$p < 0.001$
40%	10	22.10 ± 0.35	NS ($p > 0.05$)
CHX 0.2%	10	22.50 ± 0.40	Reference

CHX = chlorhexidine; SD = standard deviation. Statistical significance: $p < 0.05$ = significant (S); $p < 0.001$ = highly significant (HS); NS = not significant.

Table 5. Inhibition zones (Mean ± SD) for *Candida albicans* at different Chamomile extract concentrations

Concentration (%)	No. Isolates	Mean ± SD (mm)	Significance vs CHX
10%	10	11.40 ± 0.50	—
20%	10	17.80 ± 0.45	$p < 0.001$
30%	10	21.60 ± 0.38	$p < 0.001$
40%	10	25.40 ± 0.30	NS ($p > 0.05$)
CHX 0.2%	10	25.60 ± 0.28	Reference

CHX = chlorhexidine; SD = standard deviation. Statistical significance: $p < 0.05$ = significant (S); $p < 0.001$ = highly significant (HS); NS = not significant.

Candida albicans

For *C. albicans* (Table 5), a marked antifungal response was observed with inhibition zones ranging from 11.40 ± 0.50 mm at 10% to 25.40 ± 0.30 mm at 40%, statistically comparable to CHX (25.60 ± 0.28 mm). The strong fungistatic action is likely due to Chamomile's azulenes, spiroethers, and coumarins, which interfere with fungal ergosterol synthesis and cell wall integrity.

3.4 Comparative ANOVA among microorganisms

As presented in Table 6, the one-way ANOVA was used to compare the inhibition zones of *S. mutans, L. acidophilus*, and *C. albicans* at each concentration of the alcoholic German Chamomile extract. At 20%, 30%, and 40% concentrations, the differences among the three microorganisms were statistically significant ($p < 0.05$) to highly significant ($p < 0.001$), indicating that the Chamomile extract did not affect all species to the same extent at these levels. In contrast, no significant difference was observed within the control group treated with 0.2% chlorhexidine, which consistently inhibited all of the tested pathogens, indicating its wide and non-selective action. The findings suggested that Chamomile extract exhibits a selective pattern of antimicrobial activity at low and medium concentrations, with the range of effect gradually increasing with increasing concentration, approaching the efficacy of Chlorhexidine; however, depending on the target microorganism.

Figure 3 shows the zones of inhibition (mm) caused by the alcoholic extract of German Chamomile against the three oral pathogens at concentrations of 10%, 20%, 30%, and 40% compared to the inhibitory effect of 0.2% chlorhexidine. The extract showed a clear pattern of concentration-dependent inhibition across all strains tested; *C. albicans* showed the highest inhibitory response, followed by *S. mutans*, while *L. acidophilus* was the least susceptible. The extract demonstrated a clear concentration-dependent effect across all tested isolates, exhibiting the highest inhibitory response against *C. albicans*, followed by *S. mutans*, while *L. acidophilus* was the least sensitive. The extract's efficacy at a 40% concentration was comparable to that of chlorhexidine, exhibiting similar inhibitory performance. These results reinforce the broad-spectrum, dose-dependent antimicrobial activity of Chamomile extract, highlighting its potential as an effective natural option for combating bacterial and fungal pathogens in the mouth. These results further reinforce the broad-spectrum, dose-dependent antimicrobial activity of German Chamomile extract, supporting its potential as an effective natural option for combating bacterial and fungal pathogens in the mouth.

Table 6. One-way ANOVA comparing inhibition zones of *S. mutans*, *L. acidophilus*, and *C. albicans*

Concentration (%)	F-value	p-value	Statistical Significance
10%	5.412	0.009	S ($p < 0.05$)
20%	36.139	0.000	HS ($p < 0.001$)
30%	12.491	0.000	HS ($p < 0.001$)
40%	9.000	0.001	S ($p < 0.05$)
CHX 0.2%	0.036	0.965	NS ($p > 0.05$)

CHX = chlorhexidine; F = F-value; SD = standard deviation. Statistical significance: $p < 0.05$ = significant (S); $p < 0.001$ = highly significant (HS) ; NS = not significant.

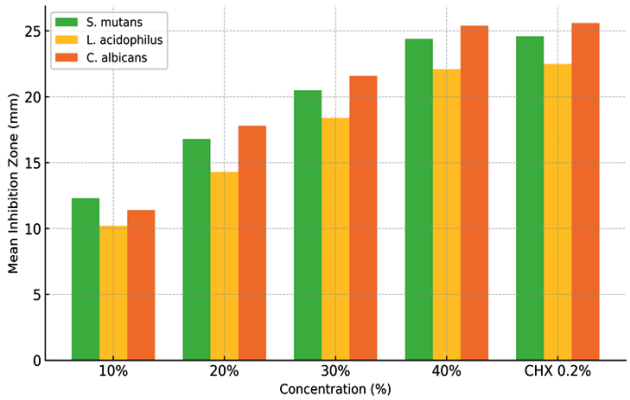

Figure 3. Comparative effect of German Chamomile extract on oral pathogens (*Streptococcus mutans*, *Lactobacillus acidophilus*, and *Candida albicans*)

3.5 Regression equation and R^2 value

The dose-response curve for microorganisms showed a marked increase in the diameter of the inhibition zone as the concentration of Chamomile extract increased from 10% to 40%, reflecting a dose-dependent effect. The value of R^2 recorded 0.998, 0.999, and 0.981 for *S. mutans, L. acidophilis,* and *C. albicans,* respectively (Figure 4).

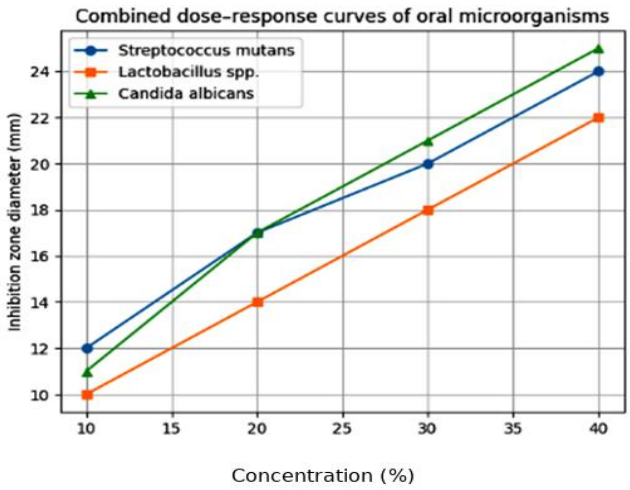

Figure 4. Dose–response curves of Chamomile extract against oral microorganisms

3.6 MIC, MBC, and MFC

The alcoholic extract of Chamomile had a MIC between 1.25% and 5%, and also has an MBC and minimum fungicidal concentration MFC between 2.5% and 10%. The extract showed strong antimicrobial activity at fairly low levels; the lowest MIC and MFC against *C. albicans* was 2.5%, 1.25% which shows that it is very good at killing fungal cells. *S. mutans* was moderately sensitive to the extract at an MIC and MBC concentration of 5.0%, 2.5%, whereas *L. acidophilus* necessitated a higher concentration of 10.0% and 5% for inhibitory and complete lethality. These results show that the extract has an effect on both bacteria and fungi, with a stronger effect on fungal cells than on bacterial cells. This strengthens its potential as a promising natural antimicrobial.

The variation in the slope of the regression showed that *C. albicans* was most affected by increasing extract concentrations. This is attributed to the nature of the fungal membrane, which is rich in ergosterol and therefore more susceptible to the terpene and phenolic compounds of Chamomile, compared to the thick cell wall of Gram-positive bacteria. The high R^2 values also confirm that the antimicrobial effect of the extract was consistent and predictable within the range of concentrations studied.

The present study demonstrates that the alcoholic extract of German Chamomile exerts marked antimicrobial activity against *S. mutans, L. acidophilus*, and *C. albicans*. The inhibition zones increased proportionally with extract concentration, and at 40%, the effect was near the control, which is 0.2% chlorhexidine, the current standard antiseptic in dentistry. This similarity is clinically relevant, since chlorhexidine, despite its broad antimicrobial action, commonly causes tooth staining, mucosal irritation, and taste disturbance [27, 28]. The ability of Chamomile to achieve comparable microbial inhibitions without similar side-effects supports its use as a natural, well-tolerated alternative in oral hygiene formulations, which has been studied and confirmed by some controlled clinical studies. Goes et al. [28] reported that *Matricaria chamomilla* mouthwash showed reductions in plaque and gingival indices, and none of the participants in the Chamomile group experienced discoloration or taste alteration associated with chlorhexidine use.

GC-MS analysis identified α-bisabolol, chamazulene, spiroethers, apigenin, luteolin, and coumarins as dominant phytochemicals. These constituents have been frequently suggested to be associated with the pharmacological properties of Chamomile, based on available experimental evidence [29]. α-Bisabolol, a sesquiterpene alcohol, is known to penetrate microbial lipid bilayers and increase membrane permeability, producing cytoplasmic leakage and cell death. Apigenin and luteolin inhibit *S. mutans* glucosyltransferase and F-ATPase enzymes, which are essential for acid tolerance and biofilm development [30]. Coumarins interfere with DNA gyrase and topoisomerase, while chamazulene modulates oxidative and inflammatory pathways [31]. These terpenoid and flavonoid compounds are known to possess potent antimicrobial activity, which may have contributed to the broad-spectrum, concentration-dependent inhibition observed in both bacterial and fungal isolates [32].

The potent antifungal properties demonstrated that Chamomile-based formulations may serve as an effective treatment for oral candidiasis. This may be particularly beneficial for individuals who cannot tolerate prolonged courses of azole therapy, as it offers a milder and potentially more effective alternative. The results of this study are consistent with previous research indicating that various components of Chamomile, especially terpenoids and their coumarin derivatives, disrupt fungal sterol synthesis, impair cell membrane integrity, and prevent yeast migration to fungal hyphae, a process typically associated with fungal development [33].

These results provide preliminary evidence supporting the antimicrobial potential of Chamomile extract as a natural agent for treating oral infections. Streptococcus mutans showed significant sensitivity, producing a 24.4 mm diameter inhibition zone at a 40% concentration, comparable to that of chlorhexidine, indicating potent activity against caries-causing streptococci. Similar inhibitory effects of Chamomile extracts against oral pathogens have been previously reported [34, 35]. In contrast, acidophilus lactobacilli showed moderate sensitivity, which can be attributed to their acid tolerance and the structural resistance conferred by their thick peptidoglycan cell wall. However, higher concentrations of the extract still produced measurable inhibition, confirming a broad antimicrobial effect. Similar results have been documented for lactobacilli species, whose acidic physiological properties enhance their resistance to plant-derived phenolic compounds [36]. Furthermore, Chamomile-containing mouthwash formulations have shown a reduction in the numbers of Streptococcus mutans, Streptococcus sobrinus, and Lactobacillus acidophilus bacteria comparable to the effect of chlorhexidine [37].

In addition to its antimicrobial activity, Chamomile also exhibits anti-inflammatory effects. Its high flavonoid content has been associated with reduced levels of inflammatory mediators such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), contributing to improved gingival healing and mucosal protection [29, 38]. This dual antimicrobial and anti-inflammatory effect may offer therapeutic benefits for oral health.

Statistical analysis (ANOVA) revealed significant differences in microbial susceptibility, which likely reflect differences in cell wall permeability and structural composition among microorganisms [39]. The extract's activity may involve multiple mechanisms, including membrane disruption, enzyme inhibition, interference with quorum sensing, and suppression of biofilm formation, along with antioxidant effects that reduce oxidative stress [38, 40].

The stronger antifungal activity observed against *Candida albicans* may be due to increased membrane permeability, the generation of reactive oxygen species, and the presence of fungal-specific targets such as ergosterol and chitin. In contrast, bacterial peptidoglycan layers and dense biofilm formation provide relative protection against plant compounds [40-42].

Although these in vitro findings demonstrate promising antimicrobial activity, they may not fully reflect the complexity of the oral environment. Factors such as salivary flow, microbial interactions, diet, and host immunity can influence the results; therefore, further animal studies are needed to confirm the clinical applicability of Chamomile extract.

4. CONCLUSIONS

The results of this study showed that the alcoholic extract of German Chamomile possesses antimicrobial activity against several important oral microorganisms, notably *S. mutans*, *L. acidophilus*, and *C. albicans*. Gas Chromatography–Mass Spectrometry (GC-MS) analysis also revealed that the extract contains a mixture of bioactive compounds, including alpha-bisabolol, chamazulene, apigenin, and luteolin. These are probably what caused most of the activity seen in the tests. There was a clear dose-related pattern in all of the tests. *C. albicans* reacted the most strongly to the extract, followed by *S. mutans* and then *L. acidophilus*. Notably, at concentrations between 20% and 40%, the inhibition zones produced by the extract were very close to those achieved with 0.2% chlorhexidine, suggesting that Chamomile may hold meaningful therapeutic value in oral care settings. Therefore, chamomilla extract can be considered a promising natural alternative to conventional chemical antiseptics for oral care applications, offering effective control of bacterial and fungal biofilms with potentially fewer side effects. Further in vivo and formulation-based studies are recommended to validate its clinical applicability and safety profile.

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NOMENCLATURE

ANOVA	Analysis of Variance
BHIA	Brain Heart Infusion Agar
BHIB	Brain Heart Infusion Broth
CFU	Colony Forming Unit
CHX	Chlorhexidine
GC–MS	Gas Chromatography–Mass Spectrometry
g	Gram
MBC	Minimum bactericidal concentration
MFC	Minimum fungicidal concentration
MIC	Minimum bactericidal concentration
ml	Milliliter
mm	Millimeter
MSBA	Mitis Salivarius Bacitracin agar
R^2	Coefficient of determination
SDA	Sabouraud Dextrose Agar
SD	Standard deviation
SPSS	Statistical Package for the Social Sciences
UK	United Kingdom
w/w	Weight-by-weight